NANO-FABRICATED SIZE EXCLUSION CHROMATOGRAPH

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Abstract
This paper describes the development of a nano-fabricated size exclusion chromatograph (nSEC) based on the principle that molecules traveling through a microcolumn containing nano-fabricated features will have characteristic elution times that directly correlate to molecular weight. Compared to conventional size exclusion chromatography, the nSEC offers greater control over the size exclusion process; mass fabrication; integration of the separation column with associated valves, pumps, and detectors; and dramatic reductions in instrument mass and power requirements.

Keywords: nanofluidics, lab-on-a-chip, chromatography, separation

1. Introduction

Size exclusion chromatography is a subset of high-pressure liquid chromatography (HPLC) in which molecules are separated based on their retention time in a size exclusion column consisting of small (~10μm) closely packed silica or polymer beads with uniform nanopores ranging from 10 to 1000nm [1]. Molecules larger than the exclusion limit are unable to enter the nanopores and remain in the liquid mobile phase exiting the column first. Molecules smaller than the permeation limit can completely penetrate the nanopores, are retained in the stationary phase, and elute last. Fractionation occurs between intermediate sized molecules as the ratio of analyte concentration in the stationary phase to that in the mobile phase decreases with increasing molecular size. Thus molecules elute in order of decreasing size and can be detected with a variety of methods, including laser-induced fluorescence. The resulting chromatogram yields molecular identification based on peak elution times and molecular concentrations that can be inferred from the peak size.

2. Device Description and Fabrication

The nSEC's nano-fabricated features, analogous to the traditional SEC's bead nanopores, consist of size-exclusion gaps defined in the z-direction over a matrix of microchannels in the x-y plane, similar to the interstices between the beads (Figure 1).

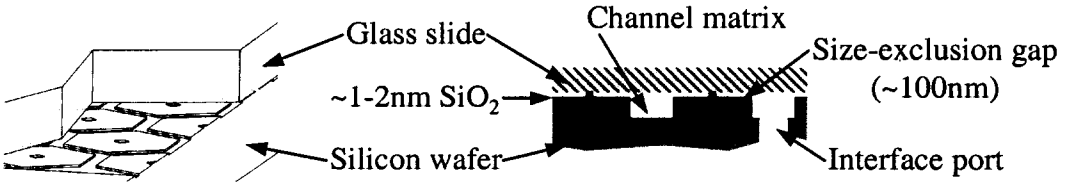

Figure 1: 3-D and cross-sectional views of nSEC schematic

Ion milling is used to define the 100nm tall support posts in silicon, followed by reactive ion etching of the channel matrix (3μm wide x 0.4μm deep) (Figure 2). A ~1-2nm thermal oxide layer is grown over the entire wafer to create a chemically uniform surface. Interface ports are water-jet drilled and finally, a co-efficient of thermal expansion matching cover glass is anodically bonded to the micromachined wafer forming the device roof.

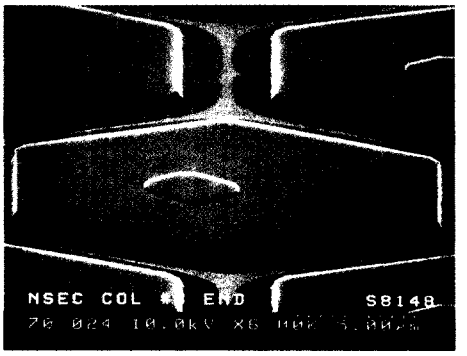

Figure 2: nSEC channel matrix and 100nm tall support posts

3. Theoretical Model

We have developed a geometric model for nSEC separations of an analyte sample of spheres based on the diffusion of the analyte molecules between the eluent flow in the microchannel matrix and the eluent flow in the size-exclusion gap. The eluent flow rate in the gap is negligible compared that in the channel matrix and is considered to be zero. The diffusion distribution constant, K_i, describes each molecule's probability of entering the size exclusion gap. The constant for planar slab pores is used [2];

$$K_i = 1 - \frac{r_i}{a}, \tag{1}$$

where r_i is the radius of the molecular radii and a is half the planar gap. It is the differences in the diffusion distribution constant that lead to analyte separation. As the analyte plug, introduced at the beginning of the column, passes through nSEC, the analyte concentration in the mobile phase, c_m, decreases due to dilution by the fraction of molecules able to go into the gap. The concentration of molecules in the stationary phase, c_s, is that fraction of molecules in the mobile phase that can enter the gap:

$$c_m = \frac{V_m c_i}{V_s K_i + V_m}, \quad c_s = K_i c_m; \tag{2), (3}$$

where V_m is the mobile phase volume, V_s is the stationary phase volume, and c_i is the initial molecule concentration. Diffusion between the mobile and stationary phases as the eluent pushes the molecules through the column, broadens the initial length, ΔL_{ini} of the analyte plug. Assuming a triangular peak at the column exit, the exit band length in terms of time, Δt, is determined by mass balance,

$$\frac{c_{\max}}{2}(a_m u \Delta t) = \frac{c_s V_s \Delta L_{ini}}{L_c}, \quad c_{\max} = c_s. \tag{4}$$

In the above equations, c_{max} is the maximum concentration of the peak equal to the analyte concentration in the stationary phase, a_m is the cross-sectional area of the matrix channels, u is the mobile phase velocity, and L_c is the length of the column. Retention time, t_r, at which the analyte band exits the column, is inversely proportional to the velocity of the analyte band, which can be determined by the mass fraction, m_f, the ratio of analyte mass in the stationary phase to the analyte mass in the mobile phase [2]:

$$m_f = \frac{c_s V_s}{c_m V_m}, \quad t_{r,i} = \frac{L_c}{\left(\frac{u}{1+m_f}\right)} \qquad (5), (6)$$

A normal Gaussian distribution of the analyte concentration in the stationary phase, $c_{s,i}$, at $t_{r,i}$ over Δt approximates the chromatogram. We have applied this model to the size exclusion analysis of a water sample containing a variety of nanospheres. Assuming the initial sizes and concentrations given in Table 1, a size-exclusion gap of 100nm, a sample volume of 0.036pL, a flow rate of 0.0015nL/min (@55 psi), and negligible surface interactions, our model yields the chromatogram shown in Figure 3.

Table 1: Modeled sample diameters and concentrations

Analyte Diameter (nm)	Initial Concentration (c_{ini})
2	0.1
4	0.2
8	0.1
44	0.05
100	0.06

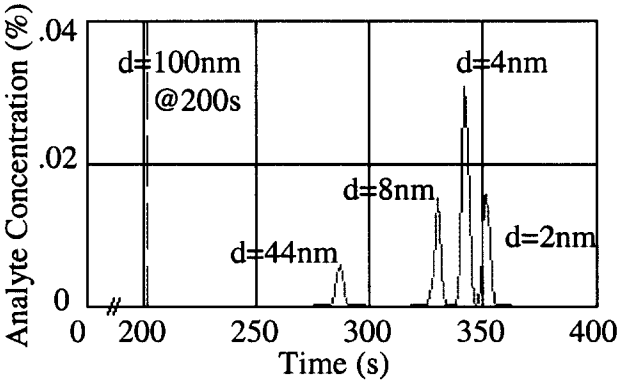

Figure 3: Chromatogram of modeled nSEC separation

5. Conclusions

We are currently developing a novel nano-fabricated size exclusion chromatograph and have completed initial fabrication and modeling. Development of the nSEC will continue with a constant flow rate, nanofluidic injection system consisting of a coupled-syringe pump driven by AFM-positioning motors. Initial testing will focus on size exclusion separations of fluorescently labeled polystyrene nanobeads. With its relatively simple fabrication process incorporating precise control over nano-size features in a chemically uniform, easily modified SiO_2 surface, the nSEC may offer highly sensitive separations of a variety of analytes, including lipids, proteins, and peptides, in an integrated hand-held device.

Acknowledgements

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References

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